EFFECT OF DIFFERENT BIOMASS CONCENTRATION ON BIOLOGICAL PRETREATMENT TO CHEMICAL PULPING PROCESS

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I declare that this thesis entitled "*Effect of Different Biomass Concentration on Biological Pretreatment to Chemical Pulping Process*"is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Date : 4 November 2006

DEDICATION

Special dedication to my mum,Fatimah Hj Ismail; my dad, Hamidi Abd Rashid; and family members that always inspire, love and stand besides me, my supervisor,Mrs Nor Azwina Zainol; my beloved friends, Jessica, Siti Aishah, Norulshahida, Hafizah, Marsilla, Nadzmiah; my fellow colleagues,
and all faculty members

For all your love, care, support, and believe in me.Thank you so much.

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ABSTRACT

Biological pulping (biological pretreatment) has been an area where it has been stated that there is a need for a “breakthrough technology”. Biological pulping is the treatment of lignocellulosic materials with oxidative lignin-degrading microorganism enzymes prior to pulping process. The aim of this study is to determine the effect of different biomass concentration on biological pretreatment to chemical pulping process. By using three different concentrations of biomass (B1, B2 and B3) banana stem waste will be treated through biological pretreatment. Treated banana stem waste will be proceeding to chemical pulping process. Untreated banana stem waste as control will also experienced chemical pulping process through the same conditions. Compositions of lignin, cellulose and glucose have been analyzed before and after biological pretreatment and after chemical pulping process by using different method and procedures. From this study, the higher biomass concentration gives the higher percentage of lignin degradation. Biological pretreatment shows the positive response to chemical pulping process where banana stem waste that have been treat in biological pretreatment process degrade the lignin in the short period of time in chemical pulping process where it takes only 30 minutes to degrade the lignin compare to 2 hours chemical pulping processes that have been stated in the literature review. This result shows that biological pretreatment helps to reduce time and energy consumption in chemical pulping process.

ABSTRAK

Proses pra-rawatan biologikal merupakan satu alternatif baru yang diperlukan dalam proses "pembaharuan teknologi". Proses pempulpaan biologikal adalah rawatan terhadap bahan lignoselulosik dengan menggunakan enzim daripada mikroorganisma yang bersesuaian dengan proses pempulpaan. Tujuan kajian ini adalah untuk mengenal pasti pengaruh kepekatan biojisim yang berlainan terhadap pra-rawatan biologikal kepada proses pempulpaan kimia. Dengan menggunakan kepekatan biojisim yang berlainan (B1, B2 dan B3) sisa batang pisang akan dirawat melalui proses pra-rawatan biologikal. Selepas proses pra-rawatan biologikal, sisa batang pisang yang telah dirawat kemudiannya akan melalui proses pempulpaan kimia. Sisa batang pisang yang tidak dirawat juga akan melalui proses pempulpaan kimia melalui kaedah yang sama. Komposisi lignin, selulosa dan glukosa akan dianalisa sebelum dan selepas proses pra-rawatan biologikal serta proses pempulpaan kimia. Daripada kajian yang telah dijalankan, didapati kepekatan biojisim yang paling tinggi menghasilkan pengurangan lignin yang paling tinggi. Daripada kajian ini juga, didapati pra-rawatan biologikal telah membantu menjimatkan masa dan tenaga dalam proses pempulpaan kimia dimana masa yang di ambil untuk proses pempulpaan kimia hanya 30 minit berbanding 2 jam didalam proses sebenar yang telah dinyatakan didalam rujukan.

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LIST OF SYMBOLS

kg	-	Kilograms
cm	-	Centimeter
mm	-	Millimeter
L	-	Liter
°C	-	Degree celcius
g	-	Gram
mg	-	Milligrams
µliter	-	microliter
ml	-	Milligrams
µg	-	microgram
N	-	Normality

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CHAPTER 1

INTRODUCTION

1.0 Introduction

The purpose of pulping is to extract cellulose fibers from plant material, generally hardwood, softwood trees or non wood plant for papermaking. The most abundant component of the native wood matrix is cellulose, a polysaccharide that is desired for paper production. The second most abundant component of native wood is lignin, a complex polymer made of aromatic units. Two approaches have been employed to pulping and they are chemical pulping and mechanical pulping. Mechanical pulping involves the use of mechanical force to separate the wood fibers but chemical pulping dissolves lignin from cellulose and hemicellulose fibers by using chemicals (Messner and Srebotnik, 1994).

Chemical pulping, dissolving the lignin in the raw material used to create pulp, is the most commonly used pulping process. The three main types of chemical pulping are the more common sulfate pulping (most commonly known as Kraft pulping), sulfite pulping and soda pulping (Smook, 1992). In this study, chemical pulping condition was applied after biological pretreatment.

Biological pretreatment is also known as biopulping in pulp and paper technology, appear to have potential to overcome some of the problems associated with mechanical manufactured pulp (Berrocal, 2004) and decrease chemical consumption in

chemical pulping operations (Messner and Srebotnik, 1994). Biopulping is an environmental friendly technology by reduces electrical energy consumptions and avoiding pollution by reducing the chemicals used in chemical pulping.Recent reports show that a biologically based approach has potential for improving both the economics and environmental impact of pulp generation (Scott et al., 1998).

The biopulping effect apparently is dependent on the particular raw materials, microorganism, biotreatment and pulping condition used (Oriaran et al., 1990; Dawson-Andoh et al., 1991; Chen and Schmidt, 1995). There are a lot of parameters affecting biological pretreatment and one of them is biomass concentration. Biomass concentration can be defined as the total mass of living matter within a given unit of volume. In this case, the total mass living matter was the total mass or total suspended solid of mixed culture (Scott et al., 1998).

Banana stem is one example of non wood fibers that will be used in this study as a raw material. This pseudostem is usually considered waste in the banana industry. Therefore, it makes sense to turn such waste into a useful product like paper.

1.1 Problem Statement

With the advent of Information Technology (IT), world paper consumption was expected to decrease especially with the increasing deployment of paperless communication. The opposite has instead happened. Demand for paper is more than ever, and we are left to look for more raw materials to meet the needs.

For thousands of years, men have relied on herbaceous plants for paper making. All this while, wood has been the primary fiber used to manufacture paper. Statistics have confirmed that the consumption of paper is actually on the increase worldwide (Barker et al., 1997). We need more paper. So, we need to find other alternative to reduce the used of wood fibers to be converted into paper. In fact, securing adequate raw material to satisfy the increasing paper demand has developed into a serious global environmental issue. This is because, wood, the primary raw material for paper comes from environmentally sensitive forest ecosystems. With mounting pressure to conserve forest areas, the development of new material other than wood pulp is highly sought.

The alternative for this problem is to use non woody raw material to produce paper. Over the years, studies have shown that there are many plant fibers that can replace wood pulp to produce paper. These include kenaf, sugarcane, hemp and banana (Han, 1998).

Chemical pulping give a lot of bad effects to the environment because of the high chemical and energy consumption. In industrial scale, big amount of chemical have to be used to produce paper and it also produce chemical waste that is bad for our environment. So, one alternative have been discovered to recover this entire problem and it is biological pretreatment or biopulping in pulp and paper industry.

1.2 Objective

The aim of this study is:

To determine the effect of different biomass concentration on biological pretreatment to chemical pulping process.

1.3 Scope of study

i. To determine the effect of different biomass concentration variation to biological pretreatment.

ii. To study the effects of biological pretreatment on the chemical pulping process.

iii. To analyze lignin, cellulose and glucose composition.

CHAPTER 2

LITERATURE REVIEW

2.0 Introduction

Biological pulping has been an area where it has been stated that there is a need for a "breakthrough technology" (Barker et al., 1997). To a large extent, the mechanism of biopulping is still relatively mysterious. The greatest biopulping effect, as assessed by reduction in energy consumed in subsequent refining, can be seen at a very early stage of the process. At this stage raw material looks morphologically no different to untreated raw material. This suggests that the structural components, including lignin, are still present after treatment yet much less energy are required to refine this material (Messner and Srebotnik, 1994). Although the potential for application of fungal treatment has been demonstrated, a number of engineering and process alterations must be incorporated to exploit this technology.

2.1 Raw Materials for Pulping Process

In the worldwide, around 95% of all raw materials used by the paper industry to obtain cellulose pulp consists of hardwood or softwood. The other raw materials used for this purpose are known as 'non wood' materials (Jiménez et. al., 2005). Although cellulose pulps are mainly obtained from woods, several authors report that the production of pulp from non wood resources has several advantages such as easy pulping capability, excellent fibers for the special types of paper and high-quality bleached pulp (López et al., 2000 and Navaee-Ardeh et al., 2004).

In addition, considering the current increase of the concern about forests preservation and rational use of their residues, the use of non-wood plants for obtainment of cellulose pulps contributes to decrease the use of forest wood resources. Non wood pulps can be used as such, or mixed with pulp from wood or recycled paper to obtain various products such as paper, cardboard and other lignocellulosic derivatives (Jiménez et al., 1999 and López et al., 2000).

The term "non wood" was coined to distinguish plant fibers from the two main sources of wood fibers, hardwood and softwood. Non wood or agro-based fibers are derived from selected tissues of various mono or dicotyledonous plants (Parham & Kausftimen, 1974). Non wood fibers can reduce the amount of chemicals needed for pulping as well as shorten time, thus saving energy. The high cellulose content of cotton linter (85% to 90%) compared to that of wood (35% to 49% cellulose) and the low lignin content of hemp (3%) make these non wood fibers valuable for papermaking. (Han, 1998)

Table 2.1 below shows comparison of composition between hardwood fiber and non wood fibers.

Table 2.1: Chemical Composition of Some Common Non wood fiber Compared to Wood Fiber (Han, 1998)

Materials	List of materials	Cellulose (%)	Lignin (%)
Wood	Coniferous	40-45	26-34
	Deciduous	38-49	23-30
Non wood	Rice Stalk	28-48	12-16
	Wheat straw	29-51	16-21
	Barley Stalk	31-45	14-15
	Kenaf Bast	44-57	15-19

Table 2.1 shows that non wood not just rich with cellulose fibers but the composition of lignin is also low. So, it is easy to be degraded and consumed short period of time compared to wood fibers in chemical pulping process.

Agricultural wastes constitute one of the main alternative raw materials for the pulp and paper industry (Marley, 1991; Martınez et al., 1994; Sabharwal and Young, 1996; Saikia et al., 1997). Banana stem is one example of non wood fibers and usually discarded as an agricultural waste from banana plantation in Malaysia. Banana stem has been known to be a potential cellulose source (Chandra and Adinugraha, 2002 and Meenakshi et al., 2002). Chemical composition of banana stem waste was shows in table 2.2. Cellulose is a linear and high molecular weight polymer as well as natural, renewable, and biodegradable material.

Table 2.2: Chemical compositions in Banana stem (Belgacem, 2003)

Chemical composition	Composition (% w/w with respect to o.d material)
Holocellulose	65.2
Lignin	12.7
Cellulose	40.2

Banana trees fruit once in their live. After harvesting, the banana stem is considered as a waste material. For every thirty to forty kilograms of banana sold in the market, there is 250 kilograms of waste that needs to be disposed. Improper disposal can pose a problem for the environment. Therefore, it makes sense to turn such waste into a useful product like paper (Nurul Huda, 2003) by using the concept “from waste to wealth”.

Producing paper from wood pulp could also devastate tropical rainforest and woodland due to the huge demand of the wood pulp paper. Nowadays, from the 170 million tons of papers consumed worldwide annually, 95 percent are made from wood. On the other hand, it has been estimated that almost one billion tons of banana stems are wasted and left to rot. If the banana stems are used for paper making, the world can have 100 million tones of pulp to produce paper, which is equal to half of the paper used worldwide. This will drastically reduce deforestation. Besides, for every ton of banana stem used for paper making, it can save around twenty mature trees from being cut down for paper. This can save the seven million hectares of trees from being used for wood pulp each year (Nurul Huda, 2003)

Furthermore, banana producing countries could earn extra revenue by exporting the banana fiber to developed nations as an alternative to wood pulp in paper making. This will help spur economic growth for the countries involved and save on foreign exchange through reduced spending on paper imports (Nurul Huda, 2003).

2.2 Biological Pretreatment

Biopulping is the treatment of lignocellulosic materials with oxidative lignin-degrading fungal enzymes prior to pulping process. This biological process is reputed to increase paper-strength and reduce both chemical energy consumption and environmental impact. These enzymes, which include lignin and manganese peroxidase and laccase, are responsible for the oxidative biodegradation of PAHs (anthracene, benzo[a]pyrene) (Alcade et al., 2002), (Pickard et al., 1999), (Collins et al., 1996), (Vazquez-Duhalt et al., 1994), (Bogan and Lamar, 1996), (Johannes et al., 1996), (Majcherczyk et al., 1998) into CO_2 and H_2O. Lignin peroxidase (LiP) use hydrogen peroxide to catalyze one-electron oxidations of phenolic and non-phenolic compounds leading to alkyl aryl cleavage, aromatic ring cleavage, demethylation, hydroxylation and polymerization while manganese peroxidase (MnP) catalyze the Mn-mediated oxidation of lignin and phenolic compounds.

Laccases [*p*-diphenol-dioxygen oxidoreductase] belong to the group of enzymes called blue copper oxidases that catalytically oxidise phenols (d'Acunzo, Galli and Masci, 2002) or chlorinated biphenyls with a four-electron reduction of O_2 to H_2O. Artificial substrates such as ABTS (2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid), HBT (hydroxyl benzotriazole) or violuric acid can act as mediators enabling the oxidation of non-phenolic compounds which cannot be oxidized by laccases on their own, thereby expanding the range of applications of these enzymes (d'Acunzo, Galli and Masci, 2002).

In many cases, the fungal attack is not selective to just the lignin component of wood. The cellulose is also depolymerized by the enzymes secreted by the microorganism. But to prevent cellulose from being degraded, suitable conditions and method need to be used in chemical pulping.

The use of biological pretreatment in the industrial pulping process offers the potential for significant savings in energy and in chemical compounds not only in the cooking process during chemical pulping but also in the further refining of the raw materials used (Berrocal, 2004).

In this study, biological pretreatment will be treated first to the raw material followed by chemical pulping process.

2.2.1 Biological pretreatment with Lignin Biodegradation

Lignin makes up about one-quarter to one-third of the dry mass of wood. It is the second most abundant organic compound on earth after cellulose (Glasser and Kelley, 1987) in its contribution to living terrestrial biomass (Crawford, 1981). The common structure of softwood lignin was shown in figure 2.1. Of all naturally produced organic chemicals; lignin is probably the most recalcitrant. This is consistent with its biological functions, which are to give vascular plants the rigidity they need to stand upright and to protect their structural polysaccharides (cellulose and hemicelluloses) from attack by other organisms.

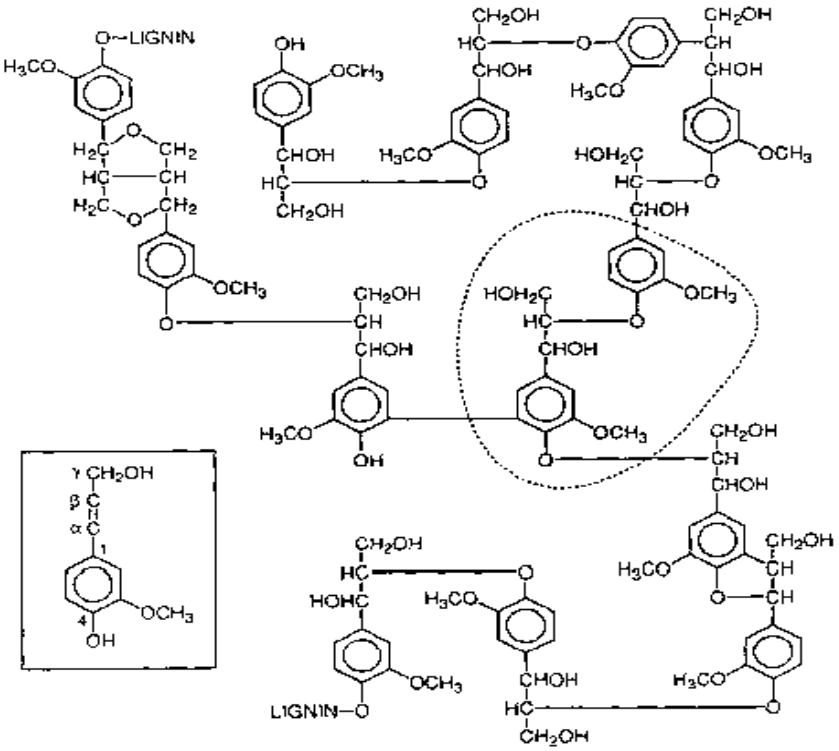

Figure 2.1: Common structure of softwood lignin (Hammel, 1997)

The cell wall of vascular plants is a highly ordered and layered arrangement of cellulose, hemicellulose and lignin. The structural of lignocellulose component was demonstrated in figure 2.2. Lignocellulose basically consists of these three components. Lignocellulose is the major structural component of woody plants and non woody plants and represents a major source of renewable organic matter. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value (Malherbe and Cloete, 2003).

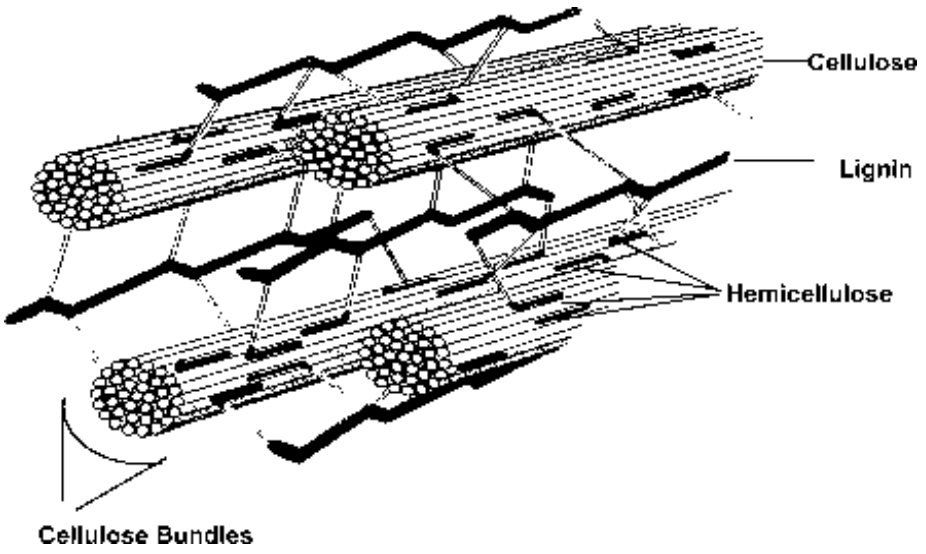

Figure 2.2: Lignocellulose (Lignin, Cellulose and Hemicellulose) (Hammel, 1997)

Large amounts of lignocellulosic "waste" are generated through forestry and agricultural practices, paper and pulp industries, timber industries and many agro industries and they pose an environmental pollution problem. Sadly, much of the lignocellulose waste is often disposed of by biomass burning, which is not restricted to developing country alone, but is considered a global phenomenon (Levine, 1996).

Cellulose $(C_6H_{10}O_5)_n$ is a long-chain polymer polysaccharide carbohydrate, of beta-glucose. It forms the primary structural component of plants and is not digestible by humans. Cellulose is a common material in plant cell walls and was first noted as such in 1838. Cellulose is the most abundant form of living terrestrial biomass (Crawford 1981). Microorganism was used in lignin biodegradation to degrade lignin and remain cellulose in plant materials.

A hemicellulose can be any of several heteropolymers (matrix polysaccharides) present in almost all cell walls along with cellulose. Their molecular weights are usually lower than that of cellulose and they have a weak undifferentiated structure compared to crystalline cellulose. But the chains form a 'ground' they bind with pectin to cellulose to

form a network of cross-linked fibers. Usually during pulping process, hemicellulose will also be degraded with lignin because of its lower amount in plant material.

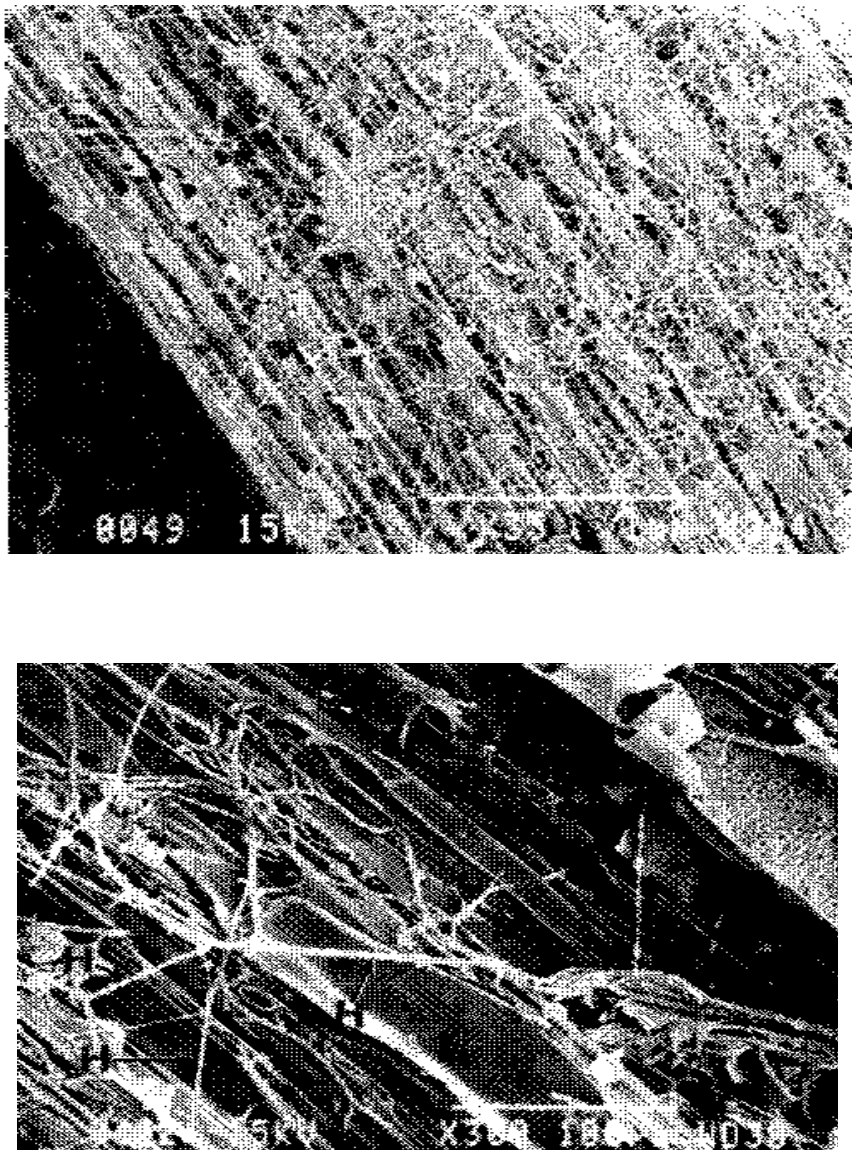

Figure 2.3: Mycelial network of *Phanerochaete chrysosporium* on surface of aspen wood chip after 3 weeks of growth (x 35) (Sachs et al.. 1989). H. hypha.

Figure 2.3 shows that the fungus, *Phanerochaete chrysosporium* has degraded the lignin after 3 weeks and remain cellulose untouched.

Biodegradation is defined as the biological catalyzed reduction in complexity of chemical compounds (Alexander, 1994). The main purpose of lignin biodegradation is to remove lignin from raw materials normally wood and non wood and the term “bio” mean degradation by using natural process. Lignin degradation is in a central position in earth’s carbon cycle, because most renewable carbon is either in lignin or in compounds protected by lignin from enzymatic degradation (cellulose and hemicellulose) (Kirk, 1983).Microorganism was used to degrade lignin rather than using chemicals in pulping process.

2.2.2 Microorganism

When vascular plants die or drop litter, lignified organic carbon is incorporated into the top layer of the soil. This recalcitrant material has to be broken down and recycled by microorganisms to maintain the earth’s carbon cycle. Were this not so, all carbon would eventually be irreversibly sequestered as lignocellulose (Hammel, 1997). Lignin biodegradation has diverse effects on soil quality. The microbial degradation of litter results in the formation of humus, and ligninolysis probably facilitates this process by promoting the release of aromatic humus precursors from the litter. These precursors include incompletely degraded lignin, flavanoids, terpenses, lignans, condensed tannins, and uberins (Hudson, 1986). Undegraded lignocellulose, e.g. in the form of straw, has a deleterious effect on soil fertility because decomposing (as opposed to already decomposed) lignocellulose supports high populations of microorganisms that may produce phytotoxic metabolites. High microbial populations in undecomposed litter also compete with crop plants for soil nitrogen and other nutrients (Lynch and Harper, 1985). By breaking down the most refractory component of litter, ligninolysis thus contributes to the removal of conditions that inhibit crop productivity.

Conditions that disfavour the biological breakdown of lignocellulose lead to soils with pronounced accumulations of litter. For example, the soils of coniferous forests in the northwest United States may contain 50 years of accumulated litterfall, because the low pH of the litter and the lack of summer rainfall inhibit microbial activity. In mature forests of this type, woody material such as dead trunks and branches can constitute 50–60% of the litter. By contrast, the soils under broadleaf forests in the eastern United States accumulate only a few years' worth of litter, and soils in some tropical rain forests accumulate virtually none, because conditions are more favourable for decomposition (Spurr and Barnes, 1980). Warm temperature, high moisture content, high oxygen availability, and high palatability of the litter to microorganisms all favour decomposition. The more highly lignified litter is, the less digestible it is, and the more its decomposition depends on the unique organisms that can degrade lignocellulose (Hammel, 1997).

No single organism is ideal for all biopulping applications. A diverse spectrum of lignocellulolytic microorganism, mainly fungi (Baldrian and Gabriel, 2003; Falcón et al., 1995) and bacteria (McCarthy, 1987; Zimmermann, 1990; Vicuňa, 1988) have been isolated and identified over the years and this list still continues to grow rapidly.

Already by 1976 an impressive collection of more than 14 000 fungi which were active against cellulose and other insoluble fibers were collected (Mandels and Sternberg, 1976). Despite the impressive collection of lignocellulolytic microorganisms only a few have been studied extensively and mostly *Trichoderma reesei* and its mutants are widely employed for the commercial production of hemicellulose and cellulose enzymes, that are hemicellulases and cellulases (Esterbauer et al., 1991; Jørgensen et al., 2003; Nieves et al., 1998). This is so, partly because *T. reesei* was one of the first cellulolytic organisms isolated in the 1950's and because extensive strain improvement and screening programs, and cellulase industrial production processes, which are extremely costly, have been developed over the years in several countries. *T. reesei*

might be a good producer of hemi-and cellulolytic enzymes but is unable to degrade lignin.

The organisms principally responsible for lignocellulose degradation are aerobic filamentous fungi, and the most rapid degraders in this group are *Basidiomycetes* (Kirk and Farrell, 1987). The ability to degrade lignocellulose efficiently is thought to be associated with a mycelial growth habit which allows the fungus to transport scarce nutrients, e.g. nitrogen and iron, over a distance into the nutrient-poor lignocellulosic substrate that constitutes its carbon source. It is curious in this regard that *Actinomycetes* (i.e. bacteria with a mycelial growth habit) have not evolved the capacity to degrade lignocellulose efficiently. It is possible that they have the ability to modify lignin somewhat, but no evidence has accumulated to show that they can degrade it (Kirk and Farrell, 1987).

The white-rot fungi belonging to the *basidiomycetes* are the most efficient and extensive lignin degraders (Akin et al., 1995; Gold and Alic, 1993) with *P. chrysosporium* being the best-studied lignin-degrading fungus producing copious amounts of a unique set of lignocellulytic enzymes. *P. chrysosporium* has drawn considerable attention as an appropriate host for the production of lignin-degrading enzymes or direct application in lignocellulose bioconversion processes (Ruggeri and Sassi, 2003; Bosco et al., 1999). Fungi selected for rapid delignification appear to be the best candidates for biopulping as they have a tendency to demonstrate selective delignification (leaving cellulose fibers untouched), as exemplified by *P. chrysosporium* (Kirk and Farrell, 1987).

2.2.3 Parameter Affecting Biological Pretreatment

There are a lot of parameters affecting biological pretreatment and they are aeration or oxygen concentration, moisture content, temperature, organic loading rate, hydraulic retention time and also biomass concentration (Hammel, 1997).

Oxygen is required for microbes to decompose organic wastes efficiently. Some decomposition occurs in the absence of oxygen (anaerobic conditions); however, the process is slow, and foul odors may develop. Because of the odor problem, composting without oxygen is not recommended in a residential setting unless the process is conducted in a fully closed system (see plastic bag method under Composting Structures). Mixing the pile once or twice a month provides the necessary oxygen and significantly hastens the composting process. A pile that is not mixed may take three to four times longer to decompose. Raising the pile off the ground allows air to be drawn through the mass as the material decomposes. Coarse materials should be placed on the bottom as the pile is built or placed in the pile and removed after the decomposition starts. Oxygen levels should be kept at 5% throughout the entire pile. Typical oxygen percents range from 6% - 16% in the pile air spaces or in the exhausted air; and 20% at the exposed portions of the pile. Failure to keep all parts of the compost pile above the 5% oxygen level will cause the pile to "go anaerobic", with the accompanying odor problems. The more oxygen, up to at least 10-12 percent, the more quickly the biodegradation will take place (Lynch and Harper, 1985).

Adequate moisture is essential for microbial activity. Dry compost will not decompose efficiently. Proper moisture encourages the growth of microorganisms that break down the organic matter into humus. Excess water can lead to anaerobic conditions which slow down the degradation process and cause foul odors. If the pile should become too wet, it must be dried out to restart the process.

Temperature of the compost pile is very important to the biological activity taking place. Low outside temperatures slow the activity down, while warmer temperatures speed up decomposition. The microbes that make up the bulk of the decomposition process fall into two categories: mesophilic, those that live and function in temperatures of 50 to 113°F, and thermophilic, those that thrive at temperatures between 113 to 158°F. A well-mixed, adequately working compost pile will heat to temperatures between 110°F and 160°F as the microbes actively feed on the organic materials. These high temperatures will help destroy weed seeds and disease organisms within the pile (Spurr and Barnes, 1980).

Bacteria have a maximum production rate depending on the type of reactor and substrate. Organic loading is one of parameters used to describe this production rate. It is the amount of organic material put into the reaction medium per time unit. The unit is g per liter day. For a given system size, higher organic loading rates generally result in lower bioconversion efficiency (BTG Biomass Technology Group, 2003).

Hydraulic Retention Time (HRT) is one of important parameter that can effect biological pretreatment. HRT is defined as a measure of the average length of time that a soluble compound remains in a constructed reactor (Wikipedia, 2006). Different HRT will give different effect to pretreatment process. The longer a substrate is kept under proper reaction conditions the more complete its degradation will become. But the reaction rate will decrease with increasing HRT. The disadvantage of a longer HRT is the increasing reactor size needed for a given amount of substrate to be treated. A shorter retention time will lead to a higher production rate per reactor volume unit, but a lower overall degradation (Wikipedia, 2006).

In this study, only one parameter will be focused and it is biomass concentration. It will be discuss more details next section. Raw materials will be treated with different biomass concentration to investigate which biomass will degrade lignin in given period of time.

2.2.3.1 Biomass Concentration

Biomass can be considered as the mass of organic material from any biological material, and by extension, any large mass of biological matter (Howard et al., 2003). A wide variety of biomass resources are available on our planet for conversion into bioproducts. These may include whole plants, plant parts (e.g. seeds, stalks, and stem), plant constituents (e.g. starch, lipids, protein and fibre), processing byproducts (distiller's grains, corn solubles), materials of marine origin and animal byproducts, municipal and industrial wastes (Smith et al., 1987). These resources can be used to create new biomaterials and this will require an intimate understanding of the composition of the raw material whether it is whole plant or constituents, so that the desired functional elements can be obtained for bioproduct production.

Biomass concentration can also defined as the total mass living matter within a given unit of volume. In this study, the total mass living matter was the total mass or total suspended solid of mixed culture. A plant can be considered as a structure that supports specialized organs. The structure consists of wood and the specialized organs include the leaves that perform photosynthesis and the roots that collect water and nutrients. Materials are transported from one side to other by sap. Plants tissue consists of 50% to 90% water. In wood, the structural parts of plants, one finds new minerals (typically, wood ashes represent less than 1% dry mass that was burned). Wood is made of cellulose, hemicellulose and lignin in variable proportions (50%, 30%, and 20%, respectively). Cellulose, a polyhexose, and hemicellulose, a polypentose, are carbohydrates. Lignin has a phenolic structure highly resistant to microorganisms (Howard et al., 2003).

Biomass considered as "waste" can potentially be converted into various different value-added products including biofuels, chemicals, and cheap energy sources for fermentation, improved animal feeds and human nutrients (Howard et al., 2003). Biomass wastes also have significant potential applications in various industries including textile and laundry, pulp and paper, and agriculture (Howard et al., 2003).

In this study, the main focus was to using biomass (mixed culture) as an agent to degrade the lignin in banana stem waste.

2.3 Chemical Pulping

Chemical pulping, dissolving the lignin in the wood to create a pulp, is the most commonly used pulping process. Chemical pulping creates higher sheet strength than mechanical pulping. The two main type of chemical pulping are the more common sulfate pulping (most commonly known as Kraft pulping) and sulfite pulping.

The Kraft process was developed in Germany in 1879 and was first applied to a Swedish mill in 1885. The resulting paper was much stronger than any paper previously made, and therefore the process was named “Kraft”, (German and Swedish for “strength”). In terms of industrial chemical modification of lignin, the Kraft pulping process is the dominant global process (Chakar and Ragauskas, 2004). The objective of any chemical pulping processes is to remove enough lignin to separate cellulose fibers one from another, producing a pulp suitable for the manufacture of paper and other related products (Pulp and Paper Manufacture, 1987).

In a conventional kraft cook, an aqueous solution of sodium hydroxide and sodium sulfide, also known as white liquor, is reacted with the raw materials in a large pressure vessel called a digester. The white liquor and the chips are heated to a cooking temperature of about 170 ˚C and are allowed to cook at that temperature for about two hours (Smook, 1992). This cooking temperature and time are usually for hardwood fibers. For non wood fibers, it is usually required less temperature and cooking time. During this treatment, the hydroxide and hydrosulfide anions react with the lignin, causing the polymer to fragment into smaller water/alkali-soluble fragments.

The fragmentation of the lignin macromolecule proceeds through the cleavage of the linkages holding the phenylpropane units together, with a concomitant generation of free phenolic hydroxyl groups (Gellerstedt and Lindfors, 1984). The presence of these hydroxyl groups increases the hydrophilicity of the lignin and the lignin fragments. As a result, the solubility of the lignin in the cooking liquor is increased. The carbon–carbon linkages, being more stable, tend to survive the pulping process.

Delignification in the kraft cook proceeds in three distinct phases (Axegard and Wiken, 1983; Gierer, 1980): the initial phase, the bulk phase, and the final or residual phase. The initial phase of delignification takes place up to a temperature of about 150 ◦C and is controlled by diffusion (Sjostrom, 1981; Gierer, 1980). The bulk phase includes the heating period from about 150–170 ◦C and the cooking treatment at 170 ◦C. The rate of delignification in the bulk phase is controlled by chemical reactions. Most of the lignin is removed in this phase. The residual or final phase, in which the rate of delignification significantly decreases, begins when about 90% of the lignin has been removed and marks the end of the cook. The selectivity in this phase is poor, and further pulping could result in significant degradation of carbohydrates. The remaining or residual lignin, typically 4–5% (by weight) at the end of a conventional softwood kraft cook, is removed via bleaching techniques (Smook, 1992). It has been suggested that the poor selectivity in the residual delignification phase may be attributed to: (i) the low reactivity of the residual lignin towards the pulping chemicals, and hence, more resistance to delignification (Gellerstedt and Lindfors, 1991); (ii) the residual lignin being chemically linked to carbohydrates and thus, resistant to delignification (Gierer and Wannstrom, 1986; Iversen and Wannstrom, 1986).

The cooking process dissolves most of the lignin and only some of the hemicellulose, leaving mostly cellulose to hold the fibers together. The digester system may be batch or continuous process. The reason Kraft pulping is economically successful is that the used cooking liquor can be recovered and reused in the chemical recovery process.

The other process of chemical pulping is sulfite pulping which created in the United State in 1867; however it was not used in a mill until 1874 by a Swedish chemist who was probably unaware of the U.S. Patent (MacDonald, 1969). Sulfite pulping produces a lighter pulp than Kraft pulping. Sulfite pulping follows many of the same steps as Kraft pulping. The major difference in Sulfite pulping is that the digester "cooks" with a mixture of H_2SO_4 (sulfuric acid) and HSO_3^- (bisulfite ion in the form of calcium, magnesium, sodium or ammonium bisulfate). The pulp continues on through the same processes as in the Kraft pulping process. However the chemicals separated from the pulp may or may not go into a recovery process. Chemical recovery in sulfite pulping is practiced only if it is economical.

CHAPTER 3

METHODOLOGY

3.0 Introduction

Production of pulp generally contributes chemical pulping process. But in this study, banana stem waste will be treated in biological pretreatment before proceeding to chemical pulping process. The lignin, cellulose and glucose composition have to been determined before and after biological pretreatment and also after chemical pulping process.

3.1 Washing and Drying

The banana stem waste first has to be washed to remove the soil. An amount of 30 kg banana stem waste is being washed in the tap water in the sieve tray. After it is washed clean, the banana stem waste is then dried outside at ambient temperature between one to two weeks. After it was dried, chips of banana crops were prepared about 10cm x10cm and 5-7 mm thickness (Belgacem et al, 2003). The banana stem is then sealed in the seal bag and stored at ambient condition before proceeding to pretreatment or for use as an untreated control.

3.2 Colonization

All mixed culture used for biomass waste was collected from banana plantation in Kuantan. The biomass waste is the mixed culture of various microorganism, microorganism, humus, and also soil around banana stem waste. About 30 kg biomass waste was collected and placed in 10 L container. 200 g of dried banana stem waste was diluted with 0.2 L of water and then placed in the container. This procedure was repeated every week until two months. This is to make sure the growth of the mixed culture in the container.

3.3 Biomass Preparation

After two months, the experiment is ready to be performed. Different biomass concentration was prepared by using following procedure:

1. 1.0 L (X_1) (Belgacem, 2003) of biomass waste in the container was taken out and placed in the oven to measure the oven dried weight (W_{od}). To find the biomass concentration, the calculation below was applied:

$$\textit{Biomass concentration, } B_1 = \frac{W_{od}\,(g)}{V_1\,(l)}$$

2. The first step was repeated by using different X (X_2 is 1.75 L and X_3 is 2.50 L)

Now, three different biomass concentrations are prepared by label B_1, B_2 and B_3.